

Jacques Alexandre HATZFELD et al.

Q2  
--16. (Amended) Multiplication process according to claim 5, characterized in that the duration of a single resting state ranges from about 1 hour to 3 years, and is in particular about 6 hours to 72 hours, and in that the duration of a single division cycle ranges from about 6 hours to 3 years, and is in particular about 6 hours to 24 hours.--

[Amend claim 17 as follows:]

--17. (Amended) Use of non-differentiated and amplified human stem cells such as are obtained by the process of claim 5 to reconstitute human blood and/or human solid tissue or organs.--

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R E M A R K S

The above changes in the claims merely place this national phase application in the same condition as it was during the international phase, with the multiple dependencies being removed.

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Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE".

Respectfully submitted,

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By



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

3. (Amended) Use of an inhibitor according to claim ~~1 or claim 2~~, in which the inhibitor of cell development is chosen from the group consisting of products of genes which control cell development with respect to cell differentiation and/or cell division, inhibitors of cycline-dependent kinases, factors which control apoptosis or ageing, and cytokines (such as interferons and TGF-b).

4. (Amended) Use of an inhibitor according to ~~any one of claims~~ claim 1 to 3, in sequential combination with an anti-inhibitor of cell proliferation, to initiate a number of cell divisions ranging from 1 to about 100, in particular 1 to about 10, and in particular to initiate a single cell division, while maintaining the non-differentiated state of stem cells, in particular human stem cells.

8. (Amended) Multiplication process according to ~~one of claims~~ claim 5 to 7, characterized in that the stem cells, in particular human cells, are present in a cell concentration of about 1 to about  $10^{10}$  cells per ml, and in particular in a concentration ranging from about  $10^3$  to about  $10^{10}$  cells per ml, and more particularly about  $10^6$  to about  $10^9$  cells per ml.

9. (Amended) Multiplication process according to ~~any one of claims~~ claim 5 to 8, characterized in that the

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inhibitor of cell development is synthesized by the stem cells, in particular human stem cells, and/or is added to the culture medium containing the stem cells, in particular human stem cells.

10. (Amended) Multiplication process according to ~~any one of claims claim 5 to 9~~, characterized in that the inhibitor of cell development is chosen from the group consisting of products of genes which control cell development with respect to cell differentiation and/or cell division, inhibitors of cycline-dependent kinases, factors which control apoptosis or ageing, and cytokines (such as interferons and TGF-b).

11. (Amended) Multiplication process according to ~~any one of claims claim 5 to 10~~, characterized in that the inhibitor of cell development is present in a low concentration in the culture medium containing the stem cells, and in particular in a concentration ranging from about  $10^{-10}$  mg/ml to 1 mg/ml.

12. (Amended) Multiplication process according to ~~any one of claims claim 5 to 11~~, characterized in that the neutralization of the effect of the inhibitor of cell development, and in particular the inhibitor of cell proliferation, present in the culture medium is effected by

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- addition to the culture medium, in a suitable amount, of an anti-inhibitor of cell proliferation, such as an anti-TGF-b, and/or

- withdrawal from the culture medium of the inhibitor of cell development, and in particular the inhibitor of cell proliferation, belonging in particular to the cytokine group.

13. (Amended) Multiplication process according to ~~any one of claims claim 5 to 12~~, characterized in that the anti-inhibitor of cell proliferation is present in a concentration ranging from about  $10^{-18}$  to about  $10^{-3}$  g/ml.

14. (Amended) Multiplication process according to ~~any one of claims claim 5 to 8~~, in which the culture medium contains hematopoietic stem cells and comprises one or more cytokines (added to the culture medium) chosen from the group consisting of interleukins and CSF, the said cytokines being present in a concentration ranging from about  $10^{-8}$  mg/ml to about 1 mg/ml, and in particular about 10 mg/ml to 0.1 mg/ml.

15. (Amended) Multiplication process according to ~~any one of claims claim 5 to 14~~, characterized in that it comprises the following stages:

a) initiation of a first cycle of division of non-differentiated embryonic or somatic stem cells in a culture medium, and in particular of hematopoietic somatic stem cells, by seeding non-differentiated embryonic or somatic stem cells

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in the resting state in a high initial cell concentration, in particular in a concentration ranging from  $10^3$  to  $10^{10}$  cells per ml, in the presence of one or more cytokines, and by neutralization of the effect of the inhibitor of cell development, and in particular the inhibitor of cell proliferation, present in the culture medium so that the above-mentioned cells leave their resting state by the initiation of a first cell division,

b) return to resting of the non-differentiated embryonic or somatic stem cells obtained in the preceding stage with the aid of an inhibitor of cell development, the said inhibitor being synthesized by the said stem cells or being added to the culture medium,

c) if appropriate washing of the non-differentiated embryonic or somatic stem cells obtained in the preceding stage in order to remove the catabolites and the inhibitor of cell development, and in particular the inhibitor of cell proliferation which may be present in the culture medium,

d) if appropriate dilution of the non-differentiated embryonic or somatic stem cells obtained in the preceding stage in order to maintain an optimum cell concentration ranging from about 100 to  $10^{10}$  cells per ml,

e) successive repetition of the cycles of division and resting described above until the amplification factor of the cells is sufficient to obtain the number of desired stem

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cells, and in particular 2 times to about  $10^{10}$  times the number of initial non-differentiated embryonic or somatic stem cells, which corresponds to a total duration of the multiplication process of about 1 day to 3 years, and in particular 1 day to 15 days,

f) stopping of the multiplication of non-differentiated embryonic or somatic stem cells to store them, use them or cause them to differentiate in vitro.

16. (Amended) Multiplication process according to ~~any one of claims claim 5 to 15~~, characterized in that the duration of a single resting state ranges from about 1 hour to 3 years, and is in particular about 6 hours to 72 hours, and in that the duration of a single division cycle ranges from about 6 hours to 3 years, and is in particular about 6 hours to 24 hours.

17. (Amended) Use of non-differentiated and amplified human stem cells such as are obtained by the process of ~~any one of claims claim 5 to 16~~ to reconstitute human blood and/or human solid tissue or organs.